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CARDIAC SYNDROME X: ASSESSMENT OF ENDOTHELIAL FUNCTION BY PERIPHERAL ARTERIAL TONOMETRY

INAUGURAL-DISSERTATION
zur Erlangung der Doktorwürde der Medizinischen Fakultät
der Universität Zürich

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1. SUMMARY

Background: Cardiac Syndrome X (CSX) is characterized by the presence of typical chest pain, a positive exercise test result and normal coronary arteries upon angiographic examination. The development of this syndrome is thought to be heterogeneous, involving many pathogenic mechanisms. Among other mechanisms, impaired endothelial function may predominantly contribute to the pathogenesis of symptoms in CSX patients.

In this work we prospectively evaluated the role of endothelial function, using non-invasive peripheral arterial tonometry, in a cohort of patients which included healthy patients, patients with coronary artery disease and patients with CSX, from Italian-speaking Switzerland.

Methods: We examined systemic endothelial function in a cohort of 37 patients: 8 healthy patients, 15 patients with coronary artery disease and 14 CSX patients. The latter were identified from the Swiss-Italian cohort of the "Italian registry of patients with Cardiac Syndrome X" ("Registro italiano dei pazienti con Sindrome X"). Endothelial function was assessed using peripheral arterial tonometry, a validated and FDA (U.S. Food and Drug Administration) approved tool for the examination of systemic and coronary endothelial function in humans [1-5].

Results: The average values for the two main indexes measured, the "Endopat index" and the "Endopat index according to Bonetti", were over the discriminatory value of 1.67 for all studied groups. A value of less than 1.67 indicates the presence of endothelial dysfunction. Statistical analysis failed to demonstrate a significant difference between studied groups (p value of 0.442 and 0.417 respectively). Larger differences could be extrapolated in some selected subgroups, but in our study, the number of patients was too small to allow statistical analysis of the significance of the results.

Conclusion: Our results, based on a relatively small sample of patients with CSX, do not show a significant difference in endothelial function, measured by peripheral arterial tonometry, between patients with CSX and patients with coronary artery disease. The interpretation of these results, which contrast with some previous studies, is not absolute. The multitude of mechanisms involved in the development of this syndrome makes investigation of this condition, and, in particular, interpretation of results yielded from small cohorts, challenging. Moreover, the impact of the methodology used for the measurement of endothelial function, and the similar prevalence of risk factors in the two “non-healthy” patient groups (coronary artery disease patients and CSX patients) may have contributed to the neutrality of the data in our study.

2. INTRODUCTION

2.1 Definition

The first data on patients, who would now be classified as suffering from Cardiac Syndrome X, were published in 1967 [6,7], although it is likely that it was Sir William Osler, who first described this condition, in 1892, in "The Principles and Practice of Medicine". Osler described a condition defined as "hysterical angina" or "pseudo-angina", whereby patients presented with typical angina, along with normal coronary arteries, demonstrated post-mortem [8].

The term "Cardiac Syndrome X" was first introduced in 1973 by Kemp [9]. Cardiac Syndrome X (CSX) is a type of microvascular angina. In contrast to coronary artery disease (CAD), which is caused by the formation of plaques that can mechanically counteract blood circulation, in vessels of relatively large calibre, microvascular angina is limited to smaller calibre arteries. Furthermore, the accompanying ischaemia is not due to the presence of atherosclerotic plaques. The decrease in blood flow is due to other mechanisms, including internal vessel wall dysfunction which leads to macro- or micro-vascular vasoconstriction, with subsequent diminished blood flow.

CSX is a rare disorder, characterized by:

1) exertion angina or pseudo-angina associated symptoms
2) electrically positive stress test results
3) no angiographic evidence of coronary disease.

Some authors consider the presence of other systemic diseases (cardiovascular related diseases and other conditions such as hypertension and diabetes mellitus) as exclusion criteria. However, not all studies concerning CSX take these factors into account [10]. A new, less restrictive, definition of CSX, was proposed by Lanza in 2007 [11]. However, in

the absence of a consensus, and in the context of this work, we will define CSX as described above, according to the classic definition.

2.2 Background

The pathogenic mechanisms of CSX are probably multiple and complex, with a predominant role played by cardiac ischaemia, a mechanism originally proposed by Cannon *et al.* in the 1980s [12] and 1990s [13], and later supported by the work of Painting *et al.* in 2002 [14]. However, the theory that proposes ischaemia as the pathophysiological cause of pain in CSX is not universally accepted, or, more precisely, ischaemia as an essential factor in the development of CSX has not been definitively proven [15].

A further possible feature, which does not exclude other contributory mechanisms, is an increased sensitivity to cardiac pain [16]. It is also evident that, among the possible mechanisms responsible for cardiac ischaemia in patients without coronary artery disease, there is not only CSX, but there are other causes, such as coronary vasospasm (vasospastic angina or Prinzmetal's angina), thrombotic phenomena with rapid spontaneous lysis of the thrombus, cocaine use, as well as inherited thrombophilia [17]. These phenomena, however, fail to account for the entire range of symptoms recorded for patients presenting with chest pain in the context of healthy coronary arteries [17].

The ischaemic origins of CSX have been attributed to microvascular abnormalities [18], resulting from endothelial dysfunction related disorders, autonomic nervous system dysfunction and occult coronary artery disease. Endothelial dysfunction appears to play a prominent role in the development of microvascular abnormalities, but is probably just one part of a multifactorial process, and it is likely that other factors such as hypertension, hypercholesterolaemia, diabetes mellitus and smoking may contribute. Moreover, it has previously been observed in other studies, that patients with endothelial dysfunction have an increased risk of adverse cardiovascular events during follow-up [19,20], further

supporting the potential involvement of the risk factors mentioned above in disease development.

Much is known about coronary endothelial function in the context of CSX [21-23]. However, the amount of data on systemic endothelial function in the context of this rare disease is minimal. Nevertheless we have strong evidence suggesting a correlation between coronary endothelial function and systemic endothelial function in various pathological processes [24,25]. Considering that the measurement of coronary endothelial function is complex and generally invasive, a method which would allow reliable function assessment, non-invasively, would be valuable. Such a method could also address questions such as, in the context of cardiac endothelial dysfunction being related to systemic endothelial dysfunction, establishing whether the reverse is also true, i.e. if systemic endothelial dysfunction is also associated with coronary endothelial dysfunction. A positive answer to this question could confirm the role of coronary endothelial dysfunction in the pathophysiology of CSX. In addition, a non-invasive and easy to use technique would be very useful for the identification of patients with endothelial dysfunction, since, as previously mentioned, these patients have a higher risk of presenting adverse cardiovascular events. In parallel, we may be able to more easily evaluate the efficacy of potential pharmacological interventions for treatment of this rare disease. Identification of an independent target (i. e. endothelial dysfunction), moreover, would be very important to restrict the indications for therapeutic (pharmacological and non-pharmacological) actions, and would also be of great benefit from an economic point of view, given the costs induced by current therapies, especially in cases where therapies are utilised in patients presenting with chest pain without underlying obstructive coronary artery disease [26].

2.3 Epidemiology

According to studies that were published several years ago, CSX was considered to be a predominantly female disease, with a female to male ratio of 7:3 [27]. However, this finding has been put into question by meta-analysis conducted in 2010, which found that the percentage of women among CSX patients was 56%, compared to 44% male patients [10]. It is therefore not clear whether the label of "female disease" is appropriate or not. More generally, it should be noted that among patients presenting with chest pain, for whom coronary angiography is performed for suspected myocardial ischaemia originating from coronary disease, about 10% of men and 40% of women show non-significant coronary artery disease [28,29].

The average age of onset of symptoms in patients suffering from CSX is close to 50 years of age [30].

The heterogeneity of definitions used in the literature to characterize CSX, as well as the lack of uniform inclusion and exclusion criteria, demonstrate the challenging nature of accurately assessing the incidence and prevalence of CSX in the general population [10]. Among patients that undergo coronary angiography, and in whom this examination yields normal results, estimated incidences of CSX vary between 3 and 11% [10].

2.4 Pathogenesis

2.4.1 The ischaemic hypothesis

As already stated previously, myocardial ischaemia probably plays a major role in the development of the symptoms and ECG changes typically found in CSX. However, this theory remains contentious, not least because the studies which have investigated this hypothesis have always been hindered by a small number of study subjects.

Some studies used myocardial scintigraphy and assessed coronary flow reserve with various techniques, such as PET, argon washout and thermodilution measurements, and identified signs of exertion-induced myocardial hypoperfusion in patients with this syndrome [15,31]. However, other studies, which used myocardial scintigraphy, transoesophageal ultrasound during stress-tests, echo-pacing and SPECT, failed to reproduce these results [30,32,33]. Moreover, the presence of left ventricular dysfunction in CSX patients was investigated, in studies using echo-pacing, SPECT and the assessment of coronary flow reserve by thermodilution measurement, without achieving consistent results [32-35]. It was therefore suggested, that given the absence of solid evidence of macroscopic ischaemia and left ventricular dysfunction, ischaemia in the context of CSX may be limited to the sub-endocardium. This hypothesis was supported by a 2002 study which used myocardial magnetic resonance technique to demonstrate that patients with CSX have decreased sub-endocardial perfusion, when compared to a control group consisting of healthy subjects [36]. Though controversial [37], these findings were supported by another myocardial magnetic resonance study, carried out in 2008, that showed evidence in favour of a dysfunction of the cardiac microcirculation in patients suffering from CSX [38]. Moreover, a study that used phosphorus magnetic resonance, carried out in 2000, demonstrated the presence of metabolic indicators, which suggest ischaemia, in some patients presenting with chest pain associated with normal coronary angiography [39]. A study investigating lipid peroxidation product levels reached a similar conclusion (higher levels correlating with myocardial ischaemia) [40, 41].

Furthermore, coronary arterioles are the major determinants of coronary resistance [42]. However, these arterioles cannot be visualized during coronary angiography [42], which hinders effective macroscopic assessment. In addition, it is likely that ischaemia is not uniform [43], which further hampers definitive ischaemia diagnosis.

In view of the mounting evidence which supports ischaemia as the basis of CSX symptoms, it is essential to consider the mechanisms that contribute to ischaemia. At least three mechanisms are thought to be involved, namely, endothelial dysfunction, altered myocardial adrenergic activity and, possibly, occult coronary artery disease.

2.4.1.1 Ischaemia from occult coronary artery disease

The 1995 study by Wiedermann *et al*. [21], based on intravascular ultrasound (IVUS), showed morphological heterogeneity of the coronary arteries in 30 patients apparently affected by CSX. Three morphological differences were observed, with a paradoxical constrictive vasomotor response to stress that was identified in two of the morphological groups. Therefore, although CSX patients have, by definition, coronary arteries that can be defined, macroscopically, as “normal”, this study demonstrated that differences exist at smaller levels, and that a majority of these patients have epicardial arteries that could be defined as “not normal”. However, this study did not address whether these abnormalities (atherosclerotic plaques, marked intimal thickening) are sufficient to explain the symptoms present in the patients. In 2004 a Sino-German team conducted a study using IVUS and intracoronary Doppler measurements, which yielded concurrent findings [44].

Another interesting study, based on coronary computed tomography, conducted by Shemesh *et al.* [45], showed an increased frequency of coronary calcification in patients with CSX , when compared to a control group of healthy patients. However, the incidence of calcification was still lower in the CSX group, when compared to a group consisting of patients with coronary artery disease. However, even in this case the relationship between study findings and clinical symptoms could not be firmly established.

Therefore, though there is evidence of a high prevalence of occult coronary artery disease in patients with CSX, these findings have not yet confirmed a clear link between cause and effect.

2.4.1.2 Ischaemia from altered myocardial adrenergic activity

A 1991 study by Montorsi *et al*. showed a heterogeneous response to adrenergic activation in distal epicardial coronary arteries among CSX patients [46]. In 1997, Lanza *et al.* also highlighted abnormal adrenergic activity in patients with CSX [47], although the results did not definitively prove that this difference was closely linked to the pathogenetic basis of CSX.

More recently, in 2005 Madaric *et al*. published another study showing an exaggerated myocardial response to adrenergic stimulation [48]. This study included 58 CSX patients and 22 control patients, showing that the incidence of a hyperdynamic cardiac response to beta-adrenergic stimulation was significantly higher in CSX patients.

These findings confirm the potential contribution of autonomic nervous system abnormalities to the pathogenesis of CSX, in the context of an ischaemic origin of the syndrome.

2.4.1.3 Ischaemia from endothelial dysfunction

Much evidence exists to support the presence of endothelial dysfunction in patients affected by CSX, though unanimous and consistent results are lacking. Several studies yielded results which could be related to this mechanism. In 1993, a prospective study with a control group (19 patients in total) demonstrated that coronary endothelium-dependent (acetylcholine stimulation) vasodilation is impaired in subjects with CSX [51]. A second prospective study, by the same team, in 1996, with a control group (16 patients), led to similar results [52]. In 1995 a correlation between high levels of plasmatic endothelin-1, a

potent vasoconstrictor, and the early appearance of chest pain was identified within a group of 40 patients affected by CSX. Simultaneously, a higher mean rate of plasmatic endothelin-1 was found in the CSX group, when compared to the control group of healthy subjects (21 patients in the control group) [53]. Other evidence in favour of coronary endothelial dysfunction in patients with CSX was reported in a 1991 study of a group of 23 patients with typical chest pain, but no macroscopic coronary artery disease. This study attempted to establish the coronary reaction to an endothelium-dependent stimulus (acetylcholine) and to an endothelium-independent stimulus (dipyridamole), and successfully identified a subset of patients (8 out of 23), in which the cause of chest pain could be attributed to endothelial dysfunction [54]. In 1999, a prospective study showed a link between high concentrations of endothelin and a reduction in coronary blood flow [55]. It should be noted however, that recent PET-CT [56-58] and cardiac magnetic resonance imaging [59,60] analyses yielded conflicting results.

2.4.2 The “non-ischaemic” hypothesis: altered pain perception

There are several studies that suggest the presence of altered pain perception in patients with CSX. Rosen *et al.* demonstrated that, in patients with CSX, activation at the cortical level (right anterior insula) can be observed with PET-CT analysis during dobutamine ultrasound stress-testing (test leading to chest pain induction, without myocardial dysfunction, in patients with CSX) [61]. This activation is also found in patients who have pain related to coronary artery disease, and would therefore be "inadequate" in patients CSX. A 2005 study hypothesized that reduced habituation, subsequent to altered central processing of painful stimuli, as opposed to increased pain sensitivity, may be a feature of CSX [62]. In addition, patients with CSX showed a tendency to present typical chest pain, even outside the context of induction of myocardial stress [63], and felt relatively more

intense pain when submitted to the same stimuli, when compared to a control group [14,16,64-66].

A combination of various causes can therefore be implicated in the development of CSX associated symptoms, including hyperactivity of cardiac nociceptors and exaggerated perception of a stimulus, at the cortical level, which normally should be inhibited at the sub-cortical level.

2.5 Diagnosis

To date, CSX remains a diagnosis of exclusion. There is no specific test to classify a patient as having CSX. Patients must have anginal pain upon exertion, transient electrocardiographic changes (ST-segment depression) during stress-testing and angiographically normal coronary arteries. As stated above, however, there is no universally accepted definition of CSX, which hinders the establishment of a standardized diagnostic procedure. Therefore CSX is a frustrating syndrome for both physicians and patients, particularly in terms of diagnosis, and therapy.

2.6 Therapy

Therapy for CSX patients has proven to be complex and challenging, and its effectiveness is limited. Conventional therapies include treatment with drugs such as beta-blockers, calcium channel blockers, nitrates, nicorandil, and hormone replacement therapy, the use of which is based on the fact that a higher prevalence of CSX exists among peri- and post-menopausal women, though none have yielded satisfactory results. This may be due to the absence of specific therapeutic targets, elucidated from reproducible pathophysiological studies [67].

Beta-blockers

Any effect of beta-blockers upon the symptoms of CSX may be due to their ability to reduce the heart rate and myocardial oxygen demand, while simultaneously increasing adrenergic tone. Though beta-blockers are the most efficient therapy currently available for the treatment of CSX, their effectiveness is highly variable, ranging from 19 to 75% [67-71].

Nitrates

The use of nitrates for controlling CSX symptoms has enjoyed only very limited success, with just above 40% of patients presenting an improvement [27]. Also, exercise tolerance in nitrate treated CSX patients does not improve, and in some cases may even show some deterioration [72-74]. Randomized studies assessing nitrate efficacy in the treatment of CSX are scarce.

Calcium channel blockers

A four-week therapy with oral nisoldipine has proven effective in controlling symptoms and improving exercise tolerance in CSX patients [75]. In contrast, an assessment of the effect of orally administered verapamil (compared to propranolol), failed to show any benefit of anticalcic therapy [76]. Studies on calcium channel blocker therapy in CSX are limited, and mostly evaluate physiological findings, rather than symptom perception data.

Nicorandil

Nicorandil was studied in the 1990s, and yielded encouraging results in small groups of patients with characteristics that are similar to those observed in CSX [77,78], although

there are no studies on the effectiveness of this potassium channel activator on symptoms in actual CSX patients.

Alpha-blockers

The efficacy of the alpha-blocker drugs doxazosin and clonidine, in the treatment of CSX has not been proven to be statistically significant [79,80].

Angiotensin converting enzyme inhibitors

The effect of angiotensin-converting enzyme inhibitor drugs looks promising. A positive effect on symptoms and electrocardiographic findings has been reported in some sub-groups of CSX patients, particularly in patients with pre-hypertension or patients with a family history of essential hypertension and documented endothelial dysfunction [68,81].

Hormone replacement therapy

Hormone therapy has been studied ever since a link was proposed between oestrogen deficiency and vasomotor instability [82]. This therapeutic approach appears to be effective, at least initially, although observational data do not seem to show a marked improvement of symptoms. However hormone therapy remains, according to some, the most valuable therapy for treating CSX [67,68,83], particularly in female peri- or post-menopausal patients. Nevertheless, the thromboembolic risk associated with this type of therapy must not be underestimated [84-87].

HMG-CoA reductase inhibitors

The positive effects of statins on endothelial function have already been demonstrated in the 1990s [88,89]. Specific studies investigating statins and CSX have also been undertaken. In a prospective study published in 2003, the positive effects of pravastatin on

exercise tolerance, electrocardiographic changes and on endothelial function were demonstrated [90]. Similar results were presented in 2004 in a prospective study on simvastatin [91]. Statins have also been studied prospectively, in combination with angiotensin converting enzyme inhibitors, with encouraging results regarding parameters such as flow mediated dilatation and improvement of the quality of life [92]. Statins, alone or in combination with other drugs, may prove effective for CSX treatment, by reducing oxidative stress [67].

Other pharmacological and non-pharmacological interventions

Metformin is another drug that has been studied (prospectively, double-blinded, with a control group) in a small group of patients presenting characteristics compatible with CSX. This study, published in 2006, showed a decrease of anginal symptoms and an improvement was observed in exertion electrocardiographic testing results, in the treated group when compared to the control group. However, given the limited number of patients in the cohort (33 in total), further investigation is required [93].

Allopurinol, another drug which reduces oxidative stress, was the focus of a study carried out between 2008 and 2010, the results of which are pending [94].

Even aminophylline was evaluated in the treatment of CSX patients, but its effectiveness seems limited [68].

Additional drugs with different mechanisms of action, such as ranolazine, could be used in the context of treating this rare syndrome [95,96].

Other therapeutic interventions are those which merely act as analgesics. Imipramine and electrostimulation therapy (TENS, spinal stimulation) yielded encouraging results in some patients [67,80]. However, these therapies work to reduce some symptoms, but fail to address the pathophysiological basis of CSX.

3. PATIENTS AND METHODS

3.1 Patients

A total of 37 patients were included in this study. We studied 14 female CSX patients who were included in the Italian Registry on Cardiac Syndrome X. One control group consisted of 15 female patients with angiographically verified atherosclerotic disease of the coronary epicardial vessels. Another control group included 8 healthy female volunteers, with no major comorbidities, who were taking no regular medication, and who presented no risk factors such as smoking, obesity, hypertension, dyslipidaemia or diabetes mellitus. All patients came from the Canton of Ticino, Switzerland.

3.2 Methods

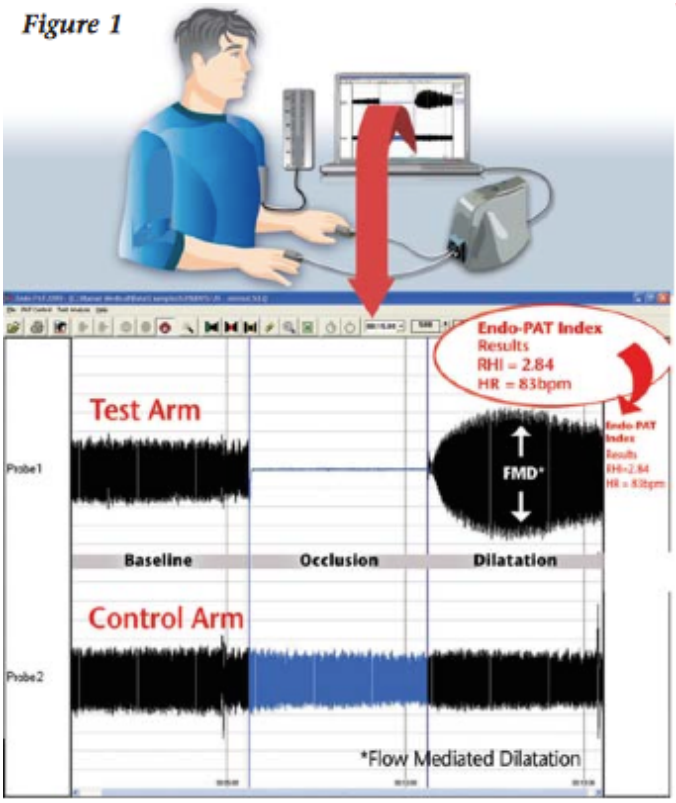
3.2.1 Study of endothelial function using a peripheral arterial tonometry device

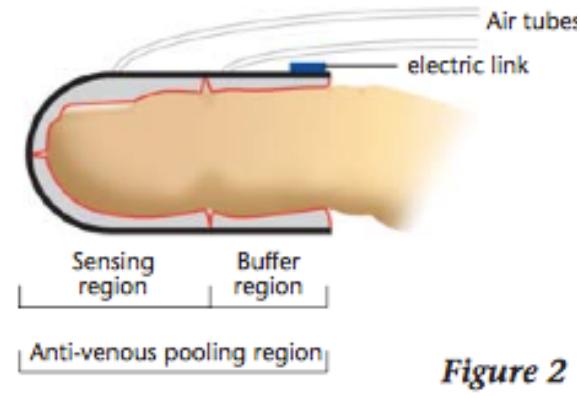
There are several clinical tests to assess endothelial function and atherosclerosis. However, most of these methods are employed to investigate the advanced stages of atherosclerotic disease, and assess anatomical or physiological vascular damage that is already evident.

However, the peripheral arterial tonometry technique is unique. This method quantifies endothelium-mediated changes in blood flow, elicited by occlusion of the brachial artery for a period of 5 minutes. The occlusion is obtained using a standard blood pressure cuff. When the cuff pressure is released, the transient increase in blood flow results in endothelium-dependent flow-mediated artery dilatation. Dilatation, which is manifested by reactive hyperaemia, is detected by the peripheral arterial tonometry device as an increase in the amplitude of the PAT (*peripheral arterial tone*) signal. For our trial, we used the EndoPAT™ device, produced by Itamar Medical Ltd, Caesarea, Israel. The pre-occlusion

to post-occlusion PAT value ratio is calculated by the software of the device, providing an RHI index (*reactive hyperaemia index*), also named the “EndoPAT index”. Software is an integral part of the EndoPAT™ device, and is used for both acquisition of data during the measurements and for analysis, post-acquisition. During data collection, the device allows real-time display of measurements. Signals are recorded to allow post-acquisition review and to enable automatic analysis of the data. Since the analysis is carried out directly by the software, the possibility of intra- or inter-operator variability is excluded. Finally, data are automatically exported to an Excel table, which includes several parameters and measurements of signal quality, and allows operators to manually calculate other indices of interest.

The PAT signal technique is used to non-invasively measure changes in the arterial blood flow in the peripheral arterial beds. The PAT signal is measured by recording pulsatile arterial volume changes at the fingertips. The EndoPAT™ device includes a measuring apparatus, connected to a pair of plethysmographic biosensors. These sensors: i) impart a sub-diastolic pressure zone at the level of the distal two thirds of the finger, thus avoiding a distal venous stasis that could cause a reflex veno-arteriolar vasoconstriction; ii) release arterial wall tension, allowing the generation of a greater dynamic range to the PAT signal; iii) reduce motion artefacts, by fixing the PAT bio-sensor to the finger.





Figures 1 and 2: from http://www.precisionhealthdiagnostics.com/Documentation/EndoPOVInternet%20October%202009.pdf

All patients were evaluated in the morning after having fasted, and avoided alcohol and tobacco for at least 8 hours. Subjects were required to rest for a 10-minute period before EndoPAT™ measurements were performed. The bio-sensors were then applied to the fingertips, bilaterally. Blood flow through the brachial artery was altered by placing a blood pressure cuff on the forearm. By inflating the cuff to between 250 and 300 mmHg, circulation distal to the cuff was stopped. After 5 minutes the pressure in the cuff was released, resulting in the restoration of flow to the brachial artery, and subsequently initiating reactive hyperaemia. Signal measurements were recorded at rest and after deflating the cuff, allowing further review of these measurements in addition to automatic computer analyses. The EndoPAT index is calculated taking into account the average

amplitude of signals recorded before cuff inflation and the average amplitude recorded during a period of between 90 and 150 seconds after cuff deflation. Test results, including multiple parameters, calculated variables and signal quality measurements were then exported to an Excel table. EndoPAT indexes <1.67 were deemed as positive for endothelial dysfunction (a value which yields a sensitivity of 82% and a specificity of 77%) [96,97]. In addition, we subsequently calculated what we call the "EndoPAT index according to Bonetti", which is an index that is determined manually. This value is a ratio between data derived from the upper member subjected to alteration of blood flow, at different time intervals, and data from the other upper member, at the same modified time intervals, without using a baseline value calculated prior to alteration of the blood flow.

Professor Piero Bonetti is the author of several seminal works on endothelial function assessment using EndoPAT™. Before commencing our prospective study, we, the operators and authors, received appropriate training at Professor Bonetti's reference laboratory. In addition, the EndoPAT™ device from our research centre was subjected to "head to head" comparison testing with the equipment from Professor Bonetti's reference centre.

4. RESULTS

4.1 Population

Our study population was divided into three groups, a group of “healthy” patients, a group of patients with proven coronary artery disease and a group of patients suffering from CSX. We carried out data analysis gathered from our three study groups.

Group	
HEALTHY	
number of patients	8
mean age (min-max)	56.75 (51-63)
CORONARY ARTERY DISEASE	
number of patients	15
mean age (min-max)	64.33 (57-72)
CSX PATIENTS	
number of patients	14
mean age (min-max)	64.86 (43-73)

Table 1: Distribution of study patients between the three groups (number of patients and mean age with range min-max)

Analysis of the results collected during our study shows that the group of healthy patients is significantly younger than the other two groups (p=.008).

Furthermore, the mean height of the group of patients with coronary artery disease was significantly lower than that of the other two groups (p=.036), and the BMI of the coronary artery disease patients was also significantly higher than the other two groups (p=.044). However, the weight (kg), did not exhibit statistically significant differences (mean weights (kg): 58.8, 67.4, 63.4; p=0.267) between the three groups.

Group	
HEALTHY	
mean height (cm)	163.8 (159-169)
mean weight (kg)	58.88 (46-68)
mean BMI (kg/m2)	21.97 (17.97-25.28)
CORONARY ARTERY DISEASE	
mean height (cm)	157.4 (140-171)
mean weight (kg)	67.40 (48-95)
mean BMI (kg/m2)	27.31 (18.75-42.22)
CSX PATIENTS	
mean height (cm)	161.6 (152-170)
mean weight (kg)	63.43 (42-88)
mean BMI (kg/m2)	24.30 (16.00-30.45)

Table 2: Mean height, weight and BMI values in all three groups (mean height, mean weight, mean BMI range min-max)

All patients included in the CSX and coronary artery disease groups followed a course of drug therapy. In the first group (CSX) 3 patients (21%) were treated with acetylsalicylic acid, 2 (14%) with clopidogrel, 8 (57%) with beta-blockers, 4 (29%) with angiotensin converting enzyme inhibitors or angiotensin receptor blockers, 8 (57%) with statins, 8

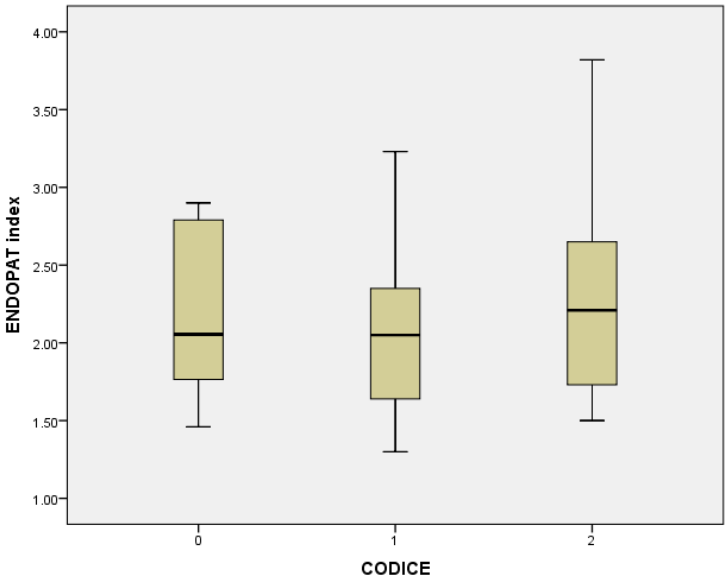
(57%) with nitrates, 9 (64%) with calcium channel blockers, 1 (7%) with aminophylline, 7 (50%) with anxiolytics, 2 (14%) with antidepressants and 3 (21%) were under hormone therapy. In the second (CAD) group, 13 patients (87%) were treated with acetylsalicylic acid, 6 (40%) with clopidogrel, 11 (73%) with beta-blockers, 9 (60%) with angiotensin converting enzyme inhibitors or angiotensin receptor blockers, 15 (100%) with statins, 0 with nitrates, 3 (20%) with calcium channel blockers, 0 with aminophylline, 2 (13%) with anxiolytics and 2 (13%) with antidepressants.

4.2 Results related to endothelial function

EndoPAT index values for the three groups are summarized in Table 3 and in Figure 3. According to our results, there were no significant differences between the three study groups (p=0.442).

Group	EndoPAT index
HEALTHY	
mean EndoPAT index	2.20 (1.46-2.90)
CORONARY ARTERY DISEASE	
mean EndoPAT index	2.07 (1.30-3.23)
CSX PATIENTS	
mean EndoPAT index	2.39 (1.50-3.82)

Table 3: Mean EndoPAT index values in the groups (mean EndoPAT index and range min-max)



Groups:

"0" : healthy patients

"1" : CAD patients

"2" : CSX patients

Figure 3: EndoPAT index (95% CI, +/- 1SD)

The same trend was found by analyzing the "EndoPAT index according to Bonetti" data (p=0.417).

Group	"EndoPAT index according to Bonetti"
HEALTHY	
mean EndoPAT index (Bonetti)	2.04 (1.24-3.59)
CORONARY ARTERY DISEASE	
mean EndoPAT index (Bonetti)	1.67 (0.90-3.00)
CSX PATIENTS	
mean EndoPAT index (Bonetti)	2.02 (1.14-3.49)

Table 4: Mean "EndoPAT index values according to Bonetti" in the three groups (mean "EndoPAT index according to Bonetti" and range min-max)

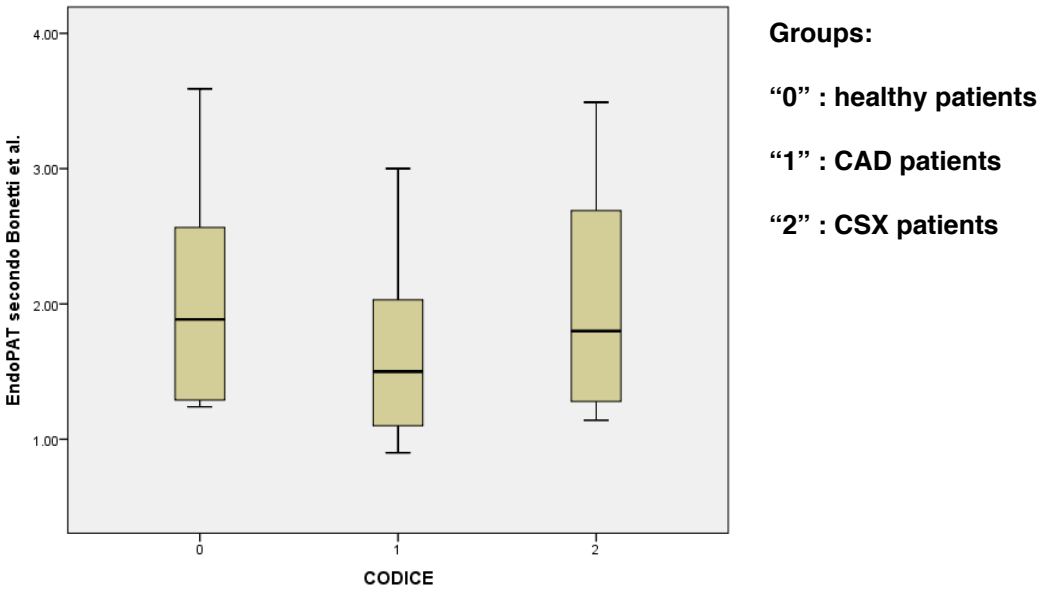


Figure 4: “EndoPAT indexes according to Bonetti” (95% CI, +/- 1SD)

We also assessed the same values (EndoPAT index and “EndoPAT index according to Bonetti”), excluding those patients who were treated with drugs that have a potential effect on endothelial function (angiotensin converting enzyme inhibitors, angiotensin receptor blockers and calcium channel blockers). Subsequent results showed that the values of both the EndoPAT index and the “EndoPAT index according to Bonetti” were found to be significantly lower, after exclusion of CSX patients who were treated with calcium channel blockers (mean value of 1.96 for the EndoPAT index and of 1.41 for the “EndoPAT index according to Bonetti”). However, the same trend was found when patients treated with nitrates were excluded from analysis (mean value of 2.14 for the EndoPAT index and of 1.80 for the “EndoPAT index according to Bonetti”). Furthermore, the average values obtained by excluding patients taking statins or angiotensin converting enzyme inhibitors are lower than those of the entire cohort, although the difference is smaller. However, at this point, the number of patients analysed (5 and 6 patients respectively) was too small to obtain statistically meaningful analysis.

5. DISCUSSION

Data that we have collected and analysed did not allow the determination of statistically significant differences between the EndoPAT index and “EndoPAT index according to Bonetti” values, obtained for the three groups of patients studied. The reliability of data of the EndoPAT index, measured only once in each patient, is proven. These values are stable and reproducible in time [99-102] and the methodology was prospectively tested by the authors of this study, against that of the reference laboratory.

The absence of significant differences in values of the calculated indexes may be due to the small cohort size. CSX is an uncommon disease, and its diagnosis is difficult and complex. Furthermore, recognition of this syndrome is not universal, even in the medical community, and therefore it may be concluded with certainty that its incidence is underestimated, which further complicates patient recruitment for studies [10,27-30]. Another confounding factor was the presence, in the coronary artery disease and CSX groups, of many patients who were following treatment regimes with drugs that affect endothelial function (statins, angiotensin converting enzyme inhibitors, angiotensin receptor blockers). It is interesting to observe that when these patients were excluded from our analysis, we observed a decrease in the average EndoPAT index values, although the groups studied in this case were, as a consequence, extremely small. The decision to perform the study without interrupting drug therapy was motivated by the desire to assess the potential of this technique for measurement of endothelial function in everyday clinical practice. Clearly, an “off medication” approach would be interesting, but is not practical for these patients who are highly symptomatic in daily life.

The study groups were significantly heterogeneous with regard to age, height, and BMI. The profusion of factors that underlie the development of CSX, along with this heterogeneity, are issues which hinder the acquisition of statistically significant results.

Another aspect which may have impacted on the results obtained is potentially inherent in the methodology used for the measurement of endothelial function, which presents, compared to other forms of measurement, such as the flow mediated dilation, greater criticality in terms of reproducibility.

The neutral results of our study could, on the other hand, clearly indicate and emphasise that the pathophysiology of CSX is complex and multifactorial and not necessarily attributable to a single pathophysiological mechanism. This is one of the reasons that prompted our group to launch a new study on CSX and on patients suffering from stress cardiomyopathy (Takotsubo syndrome) which includes, in addition to the assessment of endothelial function, investigation of various other parameters, including those related to the autonomic nervous system.

In conclusion, CSX is a rare disease whose incidence is certainly widely underestimated, and whose existence is ignored by some part of the medical community. Also, we must not underestimate the fact that patients with this syndrome have an increased risk of adverse cardiovascular effects [19,20]. The higher incidence of cardiovascular risk factors and repeated hospitalizations, caused by angina, lead to a higher number of diagnostic procedures performed, with the associated possible iatrogenic effects. Additionally, the frustration caused to both patients and physicians cannot be ignored. Patients must live with symptoms that are difficult to control, and that significantly alter their quality of life, which can prove to be a major source of stress. Clinicians are confronted firstly with diagnostic doubt, and secondly with therapies whose effectiveness are often unsatisfactory and not without side effects.

It is essential therefore for research to proceed, to simultaneously investigate pathophysiology, diagnosis and treatment. These three aspects are clearly closely related. If studies on the former are already numerous, they sometimes have lead to conflicting

results. Our study sought to test a method that is easy to perform, but potentially useful to help to elucidate the pathophysiology of CSX.

6. BIBLIOGRAPHY

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